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Characterization of acidification by the isolated perfused rat kidney: Evidence for adaptation by the distal nephron to a high bicarbonate diet

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Characterization of acidification by the isolated perfused rat kidney: Evidence for adaptation by the distal nephron to a high bicarbonate diet. To characterize acidification by the distal nephron of the intact kidney independent of alterations in buffer availability, we subjected isolated rat kidneys perfused with glucose as the sole substrate to stepwise acidification of the perfusate. In response to progressive perfusate acidification with hydrochloric acid, a maximal pH gradient between urine and perfusate, which averaged 1.71 ± 0.12, was achieved at a mean perfusate pH of 6.89 ± 0.04. The maximum pH gradient was increased when sulfuric acid rather than hydrochloric acid was used to acidify the perfusate, and it was decreased by 0.67 when amiloride (10^{-5} M) was added to the perfusate. Thus, hydrogen ion transport by the distal nephron of the intact rat kidney appears to be amenable to study, and it responds similarly to the hydrogen ion pump of anuran urinary epithelia. Kidneys from animals subjected to a variety of dietary regimens were studied in response to stepwise perfusate acidification with hydrochloric acid. Ammonium excretion averaged 0.49 ± .03 μmoles/min and did not differ significantly between any of the dietary groups. Chronic acidosis and the ingestion of either a low or high salt diet had no influence on the maximal pH gradient. Neither a low nor a high potassium diet affected the pH gradient, suggesting that the difference in urine pH between these two conditions in vivo is the result of differences in ammonia production. Ingestion of a high bicarbonate diet significantly decreased the pH gradient to 1.20 ± 0.09. Thus, an adaptive change in distal nephron hydrogen ion transport occurs in the rat kidney in response to chronic ingestion of alkali.

Caractéristiques de l'acidification par le rein de rat isolé perfusé: Preuve de l'adaptation du néphron distal à un régime riche en bicarbonate. Afin de caractériser l'acidification par le néphron distal du rein intact indépendamment des modifications de la disponibilité en tampons, des reins de rats perfusés avec du glucose comme seul substrat ont été soumis à une acidification progressive du perfusat. En réponse à l'acidification progressive du perfusat par d'acide chlorhydrique un gradient maximal de pH entre l'urine et le plasma, en moyenne de 1,71 ± 0,12, à été obtenu pour un pH moyen de perfusat de 6,89 ± 0,04. Le gradient maximum de pH à augmenté quand d'acide sulfurique à été utilisé à la place d'acide chlorhydrique pour acidifier le perfusat et à diminué quand de l'amiloride (10^{-5} M) à été ajouté au perfusat. Ainsi le transport d'ions hydrogène par le néphron distal du rein intact de rat paraît pouvoir être étudié et répondre de façon semblable à la pompe à hydrogène de l'épithélium urinaire des anuriens. Des reins d'animaux soumis à divers régimes ont été étudiés au cours de l'acidification progressive de perfusat par d'acide chlorhydrique. L'excrétion d'ammonium était en moyenne de 0,49 ± 0,03 μmoles/min et ne différait pas significativement entre les groupes. L'acidose chronique et l'ingestion d'un régime soit pauvre soit riche en sel n'a pas eu d'influence sur le gradient maximal de pH. Le contenu en potassium du régime n'a pas affecté le gradient maximal de pH ce qui suggère que la différence de pH urinaire, in vivo, entre ces deux situations est le résultat d'une différence de production d'ammoniac. L'ingestion d'un régime riche en bicarbonate diminue significativement le gradient à 1,20 ± 0,09. Ainsi une modification adaptative dans le transport d'ions hydrogène par le néphron distal survient elle dans le rein de rat en réponse à l'ingestion chronique de bases.

The final urine pH achieved by the mammalian kidney is dependent on buffer availability at the distal nephron, presumably the collecting system[1] [1, 2], the characteristics of the hydrogen ion pump [2–4], and possibly the function of a bicarbonate secretory mechanism in the collecting duct [5–6]. The major urinary buffers are bicarbonate, phosphate, and ammonia. Bicarbonate delivery to the distal nephron is dependent on its blood concentration, which determines the filtered load, and on the rate of reabsorption by the proximal portions of the nephron, which is modulated by systemic acid-base variables and the extracellular fluid volume [1]. Distal delivery of phosphate is similarly regulated, and the reabsorptive process is under the influence of several factors, including parathyroid hormone and dietary phosphorus content. The availability of ammonia is determined by the rate of renal production, which is responsive to systemic acid-base status, to other hormonal and electrolyte influences, and also to the concentration of blood glutamine [7]. Because of these multiple variables, it is difficult to obtain information about the function of the collecting system hydrogen ion pump, itself, from studies of the intact kidney in vivo.

Considerable advances in our understanding of the hydrogen ion pump have been derived from the investigation of anuran urinary membranes [3, 4], but this information cannot be extrapolated with certainty to the mammalian collecting system. Direct examination of the cortical collecting system is not

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[1]Collecting system refers to the cortical collecting tubule, and both the cortical and medullary collecting ducts. Distal nephron as used includes the distal convoluted tubule in addition to the collecting system.

feasible with micropuncture techniques; but, this segment of the rabbit nephron is accessible to in vitro microperfusion. Using this approach, some investigators have found a bicarbonate secretory capacity in this segment, which appears to adapt in response to dietary maneuvers [5, 6]; but this finding has not been confirmed by a second group [8]. In view of this controversy and because the renal acid-base mechanisms of the herbivorous rabbit differ from that of carnivorous animals [9, 10], it seems important to examine this issue in other species.

To circumvent the problems that confront investigation of distal nephron acidification in the intact animal, we developed a technique using the isolated perfused rat kidney that by both controlling and minimizing buffer availability permitted investigation, albeit indirect, of the characteristics of the distal nephron hydrogen ion pump. Using this approach, we were able to test the effect of a variety of dietary maneuvers on the distal hydrogen ion secretory mechanism. The data demonstrate that bicarbonate feeding results in an adaptation that either inhibits the distal nephron hydrogen ion pump and/or stimulates a bicarbonate secretory mechanism.

Methods

Kidneys from male Sprague-Dawley rats weighing 250 to 350 g were used. The rats were anesthetized with pentobarbital (50 mg/kg, i.p.), and they received 1 g of mannitol i.v. The ureter was cannulated with PE-10 tubing, the renal artery was cannulated with a glass cannula without ischemia, and the kidney was removed and perfused in pulsatile fashion at 37° C using a recirculating system with a reservoir as described previously [11, 12]. The perfusion medium was 7.5 g/dl dialyzed bovine serum albumin (Miles Pentex, Fraction V) in Krebs-Henseleit saline, containing 5 mM glucose, and it was filtered through a Millipore filter (0.5-μm pore size), immediately before each experiment. The perfusate (total volume, approximately 75 ml) was gassed continuously with 95% oxygen and 5% carbon dioxide, and continually filtered by Millipore filters (8-μm pore size), which were included in the circuit. Perfusate P_{CO_2} measured directly in 22 experiments at the start of perfusion averaged 33.8 ± 0.6 mm Hg and was only minimally lower after 90 min of perfusion (32.2 ± 0.7 mm Hg), and perfusate P_{CO_2} levels calculated from total carbon dioxide and pH averaged 33.9 ± 0.9 mm Hg in 48 stepwise perfusion acidification studies.

Stepwise perfusion acidification. In these experiments, the pH of the perfusate was adjusted to 7.1 by the addition of acid prior to perfusion and then decreased incrementally by the further addition of 0.2 to 0.3 mmoles of acid at 30, 50, and 70 min of perfusion. The goal was to lower perfusate pH and bicarbonate concentration sufficiently to achieve a maximal pH gradient between perfusate and urine. At each level of acidification, two 10-min urine collections were obtained, and perfusate pH was measured during the second collection approximately 17 min after addition of acid. Direct measurements of perfusate P_{CO_2} in 6 studies indicated that values at this time do not differ from measurements preceding acid addition. Perfusate bicarbonate was calculated at each pH using the P_{CO_2} determined at the start of the experiment. The lowest urine pH at each level of acidification is reported. The difference in urine pH between the two sequential specimens at the point of maximal acidification was minimal, averaging 0.13 ± 0.02 U (N = 50). Either hydrochloric acid (N = 6) or sulfuric acid (N = 6) was used to acidify the perfusate.

Effect of amiloride on urine acidification. In these studies perfusion was initiated at a pH of 6.83 ± 0.02, adjusted by the addition of either hydrochloric acid (N = 5) or sulfuric acid (N = 5). After 45 min of perfusion, amiloride (10^{-5} M) was added, and the experiment continued for another 45 min. Urine was collected at 15-min intervals during the control and amiloride periods.

The stepwise perfusion acidification and the amiloride protocols were carried out initially using normal rats fed standard laboratory chow. In a series of additional studies, kidneys from animals subjected to various dietary manipulations were studied using the stepwise perfusion acidification protocol with hydrochloric acid.

Dietary manipulations with the stepwise perfusion acidification protocol. Five groups of animals were given free access to water and fed a low sodium, low potassium diet (Nutritional Biochemicals Corporation) selectively supplemented with electrolyte. In 3 studies, the animals were given normal amounts of potassium and variable intakes of sodium and bicarbonate for 1 to 2 weeks: *(1) Low sodium (N = 6).* This diet contained sodium chloride (0.5 mmoles/100 g) and potassium chloride (6 mmoles/100 g). *(2) High sodium (N = 7).* This diet contained sodium chloride (52 mmoles/100 g) and potassium chloride (6 mmoles/100 g). *(3) Sodium bicarbonate (N = 11).* This diet contained sodium bicarbonate (52 mmoles/100 g) and potassium chloride (6 mmoles/100 g). In two studies, animals were given normal amounts of sodium and a variable intake of potassium: *(1) High potassium (N = 5).* This diet contained 90 mmoles/100 g of potassium chloride and 5 mmoles/100 g of sodium chloride, and was taken for 1 to 2 weeks. *(2) Low potassium (N = 5).* This diet contained no potassium and 5 mmoles/100 g of sodium chloride and was ingested for 19 to 20 days. A sixth group of animals (N = 5) ingested standard laboratory rat chow and had chronic metabolic acidosis produced by the ingestion of drinking water with 1.5% ammonium chloride for 1 week.

Analysis. The total carbon dioxide content of the perfusate was measured with a Natelson microgasometer. Urine and perfusate pH were measured anaerobically at 37° C. Perfusate P_{CO_2} and bicarbonate were calculated using the Henderson Hasselbalch equation with a pK'a of 6.10 and solubility coefficient of 0.03. In some instances, P_{CO_2} was measured directly with an P_{CO_2} electrode (IL model 113). Sodium and potassium were measured with a flame photometer, and ^{14}C-inulin was used for the determination of GFR. Urinary phosphate was determined according to the method of Chen, Toribara, and Warner [13]; and urinary ammonia, by adaptation of the colorimetric technique described by McCullough [14]. Paired and nonpaired Student's t tests were used for the statistical analysis.

Results

Stepwise perfusion acidification in normal animals. In the studies with hydrochloric acid, the initial perfusate pH was 7.09 ± 0.01 (bicarbonate, 8.7 ± 0.4 mM), and the urine pH averaged 5.98 ± 0.08, resulting in a mean pH gradient of 1.11 ± 0.09. As shown in Fig. 1, in response to progressive diminution of the perfusate pH, urine pH decreased further, achieving an apparent maximal gradient at perfusate pH values below 7.0. Minimal

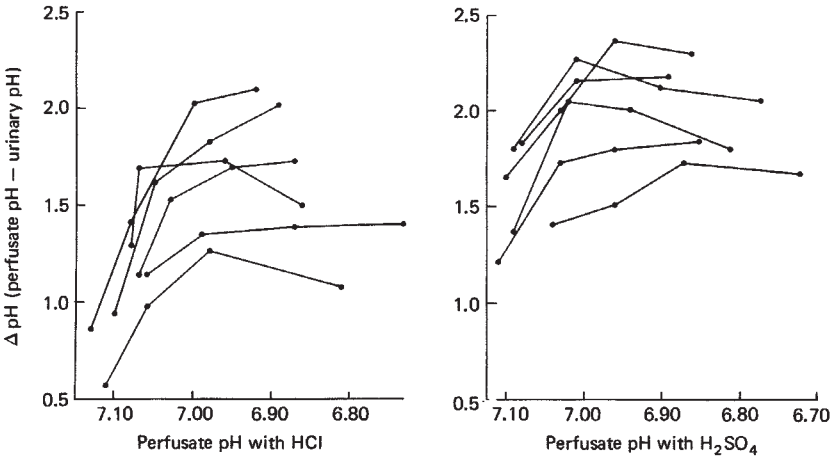

Fig. 1. *Response to progressive decrements in perfusate pH with hydrochloric acid or sulfuric acid.* With both acids, the pH gradient between urine and perfusate increased progressively as perfusate pH was decreased and appeared to achieve a maximum at a perfusate pH less than 7.0. Perfusate bicarbonate was decreased from a mean of 8.7 to 4.8 mM by the progressive addition of acid.

urine pH achieved averaged 5.18 ± 0.13 and ranged from 5.71 to 4.82. The maximal pH gradient averaged 1.71 ± 0.12 and occurred at a mean perfusate pH of 6.89 ± 0.04 (bicarbonate 5.6 ± 0.5 mM). In contrast to earlier studies at a fixed bicarbonate concentration [12], no significant correlation between sodium reabsorption and urinary acidification was found in these studies. Furthermore, unlike these initial studies [12], the present experiments were carried out without aldosterone. Thus, supraphysiologic quantities of mineralocorticoid are not required for acidification by this preparation.

As shown in Fig. 1, the response to perfusate acidification with sulfuric acid was qualitatively similar, with a maximal pH gradient also generally observed at perfusate pH values below 7.0. A quantitative comparison between the sulfuric acid and the hydrochloric acid studies, shown in Fig. 2, suggests that sulfate resulted in greater urinary acidification than did chloride. The initial perfusate pH was similar in the two groups, averaging 7.09 ± 0.01 with hydrochloric acid and 7.09 ± 0.01 with sulfuric acid; but the pH gradient was significantly greater with sulfuric acid (1.55 ± 0.09 vs. 1.11 ± 0.09, $P < 0.025$). The maximal gradient, achieved at a perfusate pH of 6.93 ± 0.03 with sulfuric acid and at 6.89 ± 0.04 with hydrochloric acid, averaged 2.07 ± 0.09 with sulfuric acid and 1.71 ± 0.12 hydrochloric acid ($P < 0.06$). The minimum urine pH achieved with sulfuric acid ranged from 4.56 to 5.05 and averaged 4.82 ± 0.08, which was significantly lower ($P < .05$) than the value with hydrochloric acid (5.18 ± 0.13). (These urine pH numbers differ slightly from those in Table 1, which refer to the urine pH at the point of maximal gradient rather than the lowest urine pH achieved.)

The renal functional parameters at the time the maximal pH gradient was achieved are given in Table 1. No difference in GFR or in fractional sodium reabsorption was found between the two studies. Presumably, the severe acidosis resulted in the low GFR found in these experiments, but sodium reabsorption was well maintained.

Effect of amiloride on urine acidification. The effect of amiloride on urinary acidification is shown in Fig. 3. In all 10 experiments, addition of amiloride to the perfusate decreased the pH gradient by an average of 0.67 ± 0.06 from a mean of 1.70 ± 0.11 to 1.04 ± 0.05 ($P < 0.01$). The change in pH gradient was striking within 15 min, and was accompanied, as expected, by a decrease in fractional potassium excretion from 0.50 ± 0.06 to 0.18 ± 0.02 ($P < 0.01$).

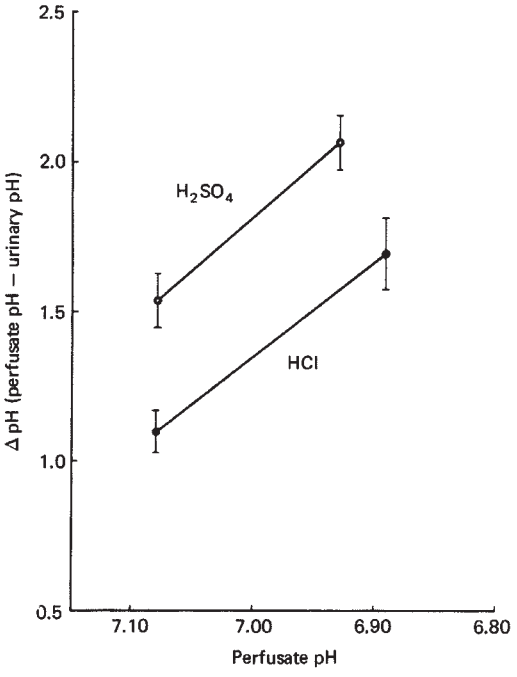

Fig. 2. *Comparison between acidification with hydrochloric acid and sulfuric acid.* The pH gradient was greater with sulfuric acid both at higher and lower perfusate pH levels. Thus, the character of the anion can influence the gradient independent of perfusate and presumably renal tubular cell acid-base status.

Influence of dietary manipulation on urine acidification: (1) Sodium and bicarbonate manipulations. As shown in Fig. 4, ingestion of a low or high salt diet has no effect on the maximal pH gradient in comparison with each other or with animals on a normal diet. Maximal pH gradient averaged 1.70 ± 0.17 on a low salt diet, 1.77 ± 0.14 on a high salt diet, and 1.71 ± 0.12 on a normal diet, as noted previously. As shown in Table 1, the degree of perfusate acidification was similar between the three groups, and there were no significant differences in renal functional parameters.

By contrast, ingestion of a high sodium bicarbonate diet resulted in strikingly different findings from ingestion of a high sodium chloride diet. Maximal pH gradient was significantly lower with sodium bicarbonate, averaging 1.20 ± 0.09 as

Table 1. Effect of dietary manipulations on acidification and renal functional parameters[a]

Diet variables[b]	Max. ΔpH	Urine pH	Perfusate pH	Lowest		Urine volume	GFR	FR_{Na}	K^+ (E/F)[c]	NH_4 excretion	Kidney dry wt
				Perfusate pH	HCO_3 mM	μl/min	ml/min	%		μmoles/min	g
Normal diet:											
With HCl perfusion	1.71 ± 0.12	5.18 ± 0.13	6.89 ± 0.04	6.85 ± 0.03	5.0 ± 0.2	21 ± 1	0.25 ± 0.05	95.6 ± 0.6	—	—	0.25 ± 0.01
H_2SO_4 perfusion	2.07 ± 0.09	4.87 ± 0.09	6.93 ± 0.02	6.82 ± 0.03	4.6 ± 0.3	22 ± 5	0.25 ± 0.03	94.2 ± 1.1			0.28 ± 0.01
P	< 0.06	NS	NS	NS	NS	NS	NS	NS			NS
$NaHCO_3$ diet	1.20 ± 0.09	5.63 ± 0.10	6.82 ± 0.03	6.79 ± 0.02	5.0 ± 0.2	87 ± 38	0.36 ± 0.12	91.4 ± 1.1	0.27 ± 0.12	0.48 ± 0.08	0.25 ± 0.01
High Na^+ diet	1.77 ± 0.14	5.11 ± 0.17	6.87 ± 0.04	6.83 ± 0.03	6.3 ± 0.8	57 ± 13	0.27 ± 0.05	91.1 ± 1.3	0.30 ± 0.05	0.46 ± 0.03	0.24 ± 0.01
P	< 0.005	< 0.025	NS	NS	NS	NS	NS	NS	NS	NS	NS
Low Na^+ diet	1.70 ± 0.17	5.20 ± 0.15	6.90 ± 0.05	6.84 ± 0.03	5.8 ± 0.5	47 ± 9	0.30 ± 0.02	94.4 ± 2.1	0.29 ± 0.06	0.48 ± 0.02	0.24 ± 0.01
NH_4Cl diet	1.76 ± 0.10	5.10 ± 0.08	6.87 ± 0.04	6.81 ± 0.02	5.8 ± 0.4	35 ± 10	0.21 ± 0.04	90.8 ± 1.4	0.49 ± 0.09	0.63 ± 0.16	0.30 ± 0.01
High K^+ diet	1.59 ± 0.11	5.23 ± 0.13	6.82 ± 0.02	6.81 ± 0.01	5.3 ± 0.4	67 ± 14	0.29 ± 0.02	90.8 ± 3.1	0.74 ± 0.11	0.45 ± 0.06	0.23 ± 0.01
Low K^+ diet	1.52 ± 0.11	5.38 ± 0.13	6.90 ± 0.03	6.80 ± 0.02	5.0 ± 0.3	60 ± 11	0.38 ± 0.05	93.0 ± 1.6	0.10 ± 0.02	0.52 ± 0.03	0.27 ± 0.01
P	NS	NS	NS	NS	NS	NS	NS	NS	< 0.005	NS	NS

[a] Values are the means ± SEM.
[b] The normal diet group was acidified with both HCl and H_2SO_4, whereas all other dietary groups underwent acidification with HCl.
[c] Excreted/Filtered

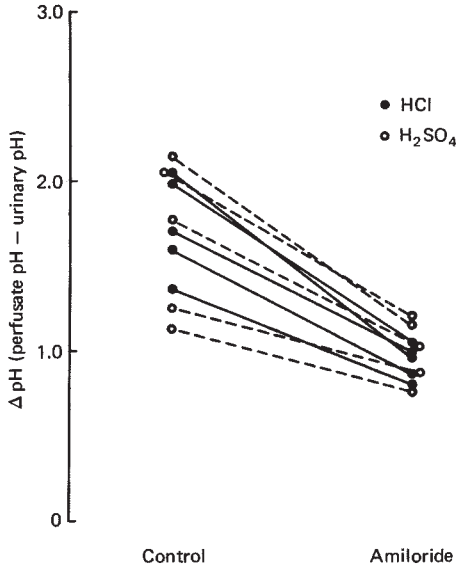

Fig. 3. *Effect of amiloride on the pH gradient.* Amiloride decreased the pH gradient when either hydrochloric acid or sulfuric acid was used to acidify the perfusate.

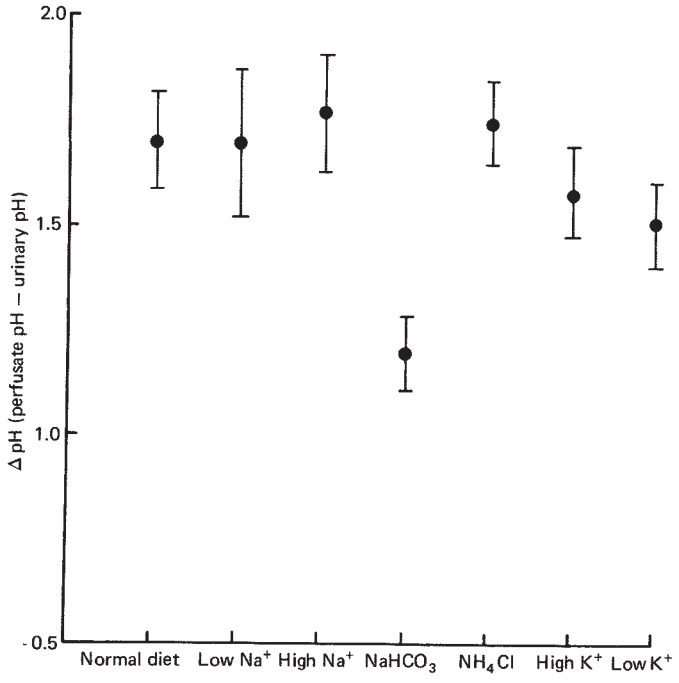

Fig. 4. *Influence of diet on urine acidification.* Kidneys from animals pre-fed a high sodium bicarbonate diet developed a significantly lower pH gradient than did kidneys from animals pre-fed a diet of comparable sodium chloride content, as well as those pre-fed the other diets shown. No significant differences were found between any of the other dietary groups.

compared with 1.77 for the high sodium chloride group. In fact, the maximal pH gradient in the bicarbonate studies was significantly lower than that achieved in all the other groups investigated. As shown in Fig. 5, this was not the result of a different qualitative response to perfusate acidification in the high sodium bicarbonate group. As in all the stepwise acidification studies, a plateau in pH gradient appeared to be achieved. Figure 5 also shows that the pH gradient with sodium bicarbonate was significantly lower than with a high sodium chloride diet at every perfusate pH value. There were no significant differences in renal functional parameters between the sodium bicarbonate and high *(sodium chloride)* groups (Table 1).

(2) Potassium manipulations. Ingestion of a low potassium diet for 3 weeks resulted in a significant potassium depletion with a decrease in muscle potassium from 477 ± 7 μmoles/g dry weight in normal rats to 339 ± 14 μmoles/g dry weight ($P < 0.005$). The maximal pH gradient did not differ between the low potassium (1.52 ± 0.11) and high potassium (1.59 ± 0.11) rats, and neither group differed significantly from the normal controls (Fig. 4, Table 1). Renal functional parameters did not differ between the potassium-depleted and potassium-adapted rats with the exception of fractional potassium excretion, which was

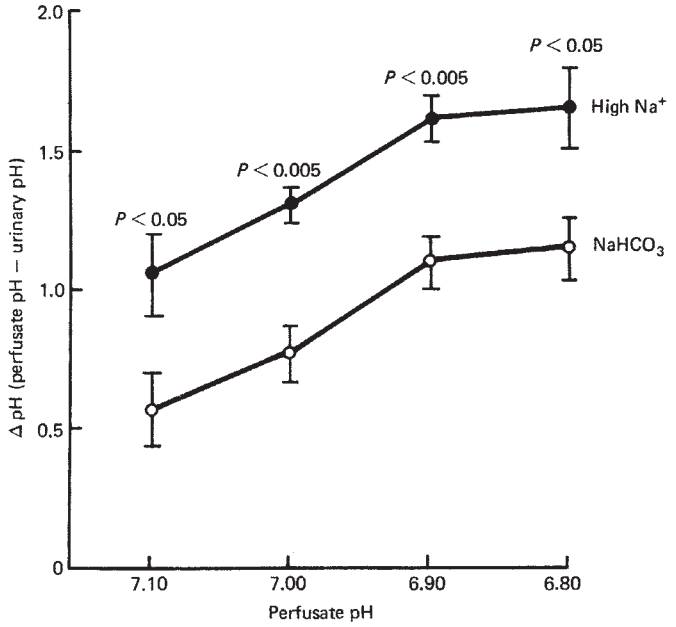

Fig. 5. *Comparison between a high sodium bicarbonate and high sodium chloride diet.* The pH gradient was significantly lower with sodium bicarbonate at every perfusate pH level. Qualitatively, the response to progressive decrements in perfusate pH was similar in both groups and a maximal pH gradient appeared to be achieved in the sodium bicarbonate studies.

significantly lower in the low potassium animals (0.10 ± 0.02 vs. 0.74 ± 0.11, $P < 0.005$).

The lower rate of potassium excretion resulted in a significantly higher perfusate potassium concentration in the low potassium studies by the completion of the experiment (5.2 ± 0.1 vs. 3.8 ± 0.2 mM). This raises the possibility that this difference in potassium concentration or potential replenishment of renal potassium store might account for the similarity in pH gradient between the low and high potassium kidneys. But, at the outset of the experiment when the potassium concentration (5.3 ± 0.1 vs. 5.0 ± 0.1 mM) and the perfusate pH (7.07 ± 0.01 vs. 7.04 ± 0.03) were similar, the pH gradients averaged 0.94 ± 0.10 in the high potassium and 1.34 ± 0.10 in the low potassium kidneys. Thus, it seems unlikely that potential replenishment of renal potassium stores resulting in a period of perfusion at a normal potassium concentration and/or differences in perfusate potassium concentration account for the similarity in acidification between the two groups.

(3) Chronic metabolic acidosis. Ingestion of ammonium chloride for 1 week did not modify the maximal pH gradient achieved, with a mean value of 1.76 ± 0.10. As shown in Table 1, the renal functional parameters with ammonium chloride did not differ significantly from the animals ingesting a normal diet.

(4) Buffer excretion. Ammonium excretion was measured in the majority of the dietary manipulation studies and did not differ significantly between any of the groups (Table 1). Mean ammonium excretion for all studies at the time of the maximal pH gradient averaged 0.49 ± 0.03 μmoles/min. Phosphate excretion, which was measured in 4 of the amiloride studies prior to addition of the inhibitor, averaged 0.052 ± 0.009 μmoles/min.

Discussion

Advantages of the isolated kidney, as compared with the in vivo kidney, are the ability to selectively control perfusate composition, to modify perfusate pH to extremes, and to eliminate the effect of hormonal and neural influences on renal function. In addition, by omitting glutamine from the perfusate, ammonia production can be minimized.

In a recent study, Tannen and Ross found that the isolated perfused rat kidney could acidify the urine effectively and that perfusate bicarbonate concentration correlated significantly with urine pH [12]. They suggested that the inability of others to demonstrate significant urine acidification utilizing this preparation [15] was the result of high bicarbonate delivery rates to the distal nephron, which masked the distal hydrogen ion secretory capacity. To decrease urine pH comparably, a requirement seems to be a lower perfusate bicarbonate concentration by the isolated as contrasted with intact kidney, presumably because bicarbonate delivery to distal sites is increased due to a modest defect in proximal reabsorption by the preparation. Although proximal bicarbonate reabsorption by the isolated perfused kidney has not been measured directly, micropuncture studies do demonstrate a diminution in fluid absorption at nephron sites between the superficial proximal and early distal convoluted tubule [16].

In the present studies, we sought to completely eliminate distal bicarbonate delivery as a significant variable affecting acidification by diminishing perfusate bicarbonate concentration to extremes. To accomplish this, we progressively decreased the bicarbonate concentration of the perfusate by the stepwise addition of hydrochloric acid. As shown in Fig. 1, kidneys from normal animals responded to progressive acidification by the development of a maximum pH gradient between urine and perfusate, which was unresponsive to further decreases in perfusate pH and bicarbonate concentration. Thus, it appeared that perfusate bicarbonate concentration could be lowered sufficiently for acidification to become independent of bicarbonate delivery to the distal nephron. Under these conditions, the pH gradient between perfusate and urine should reflect the functional characteristics of the distal nephron acidification process. This does not imply the absence of proximal nephron hydrogen ion secretion, but rather that it is no longer a rate-controlling variable.

Under free-flow micropuncture conditions, even with severe metabolic acidosis, pH values appear to be higher than 6.0 and transtubular pH gradients less than 1.0 in both superficial proximal and distal convoluted tubules [17, 18]. In addition, when distal convoluted tubules are microperfused with phosphate, extrapolated steady-state pH values are greater than 5.9 and transtubular pH gradients less than 1.1 under conditions of metabolic acidosis with a systemic pH of 7.01 [19]. Therefore, it seems highly likely that the average maximum pH gradients of 1.7 in our experiments do, at least in part, represent hydrogen ion secretion at collecting duct sites.

When the progressive acidification technique was applied in a variety of other experimental settings, the response was similar. An apparent maximal pH gradient was achieved when the bicarbonate concentration was lowered sufficiently to reduce perfusate pH to between 6.8 and 6.9. Ammonium excretion was not completely erradicated by the omission of glutamine from the perfusate, but it was minimized to a mean excretion rate of

0.49 ± 0.03 μmoles/min[2], and it did not differ significantly under a wide variety of conditions.

Phosphate excretion, which was measured in a few experiments, was only 10% of the ammonium excretion rate and, therefore, does not contribute in a significant fashion to the buffer load.

Because the influence of bicarbonate delivery to the distal nephron appears to be eliminated by this technique, and the availability of other buffers is either minimal or constant, changes in hydrogen ion gradient should reflect the intrinsic characteristics of the distal hydrogen ion pump. Based on experiments using the pH-stat technique with voltage-clamped turtle bladders, the hydrogen ion pump has been characterized both in regard to secretory capacity and proton motive force (PMF) [4]. Secretory capacity of the pump is defined by the relationship between secretory rate and the pH gradient; and PMF is delineated by the pH gradient at which hydrogen ion secretion is completely nullified. Modification in the function of the hydrogen ion pump can be characterized either by a change in secretory capacity without a change in PMF, which may reflect an altered number of pump sites, or by a concurrent change in both secretory capacity and PMF, which would imply a change in pump kinetics [4].

If buffer excretion were completely eliminated in our experiments with the isolated perfused kidney, it would be possible to equate the maximal pH gradient with the PMF of the pump. But, because this condition was not achieved despite the absence of glutamine from the perfusate, it is not possible to determine whether the pH gradient predominantly reflects the secretory capacity or the PMF of the collecting duct acidification system.

When perfused kidneys from animals fed a normal diet were subjected to acidification by sulfuric rather than hydrochloric acid, the maximal pH gradient was increased by 0.36 U. Presumably, this resulted from a more favorable potential difference (PD) for hydrogen ion transport and is consistent with the voltage dependency of hydrogen ion transport demonstrable with anuran urinary epithelia [21, 22]. Similarly, addition of amiloride inhibited the pH gradient by 0.67 U. Nullifying the transepithelial PD in turtle bladder decreases the maximal pH gradient by 0.3 U [23], and experiments investigating the effect of amiloride on toad urinary bladder suggest that a significant part of its inhibitory effect on hydrogen ion transport is related to changes in transepithelial PD [22]. Amiloride also inhibits hydrogen ion secretion by the isolated rabbit collecting duct [6, 24]. Thus, the perfused rat kidney responds to amiloride in a manner similar to the anuran urinary epithelia and to the rabbit collecting duct. These observations with sulfate and amiloride suggest that our experiments with the isolated perfused kidney reflect, at least in qualitative fashion, the transport characteristics of the distal hydrogen ion pump. Furthermore, they indicate that changes in pump function can be detected experimentally. The data with sulfate also clearly demonstrate that urinary acidification can be affected by the character of the anion independent of changes in systemic acid-base homeostasis.

Having developed an indirect technique that appears to reflect the function of hydrogen ion pump in the rat distal nephron, we then sought to determine its characteristics in response to a variety of in vivo manipulations. Ingestion of either a high or low sodium intake appeared to have no influence on distal acidification. Similarly, no alteration in acidification was apparent under conditions of chronic metabolic acidosis. But, it should be stressed that if the pH gradient largely reflects the PMF of the pump, the absence of an increase in the pH gradient does not necessarily mean that hydrogen ion secretory capacity is not increased.

When animals ingested a high bicarbonate diet for 1 to 2 weeks, the maximum pH gradient achieved was strikingly diminished. As shown in Fig. 5, the response to progressive perfusate acidification was qualitatively similar to studies on a high salt diet (and under other conditions), and a maximal gradient appeared to be achieved. Thus, the decrease in pH gradient was not the result of altered distal delivery of bicarbonate. Rather, some adaptive change in the hydrogen ion transport system of the distal nephron had transpired. This finding is reminiscent of the observation by McKinney and Burg that bicarbonate secretion by the isolated rabbit collecting duct is stimulated in animals fed a high bicarbonate diet [5]; but, for reasons that are not clear, this finding has not been confirmed by other investigators [8]. Although our data clearly demonstrate that a change in hydrogen ion transport has occurred, it is not possible at this time to determine whether a change in the PMF or secretory capacity of a hydrogen ion pump accounts for our findings, and/or whether secretion by a bicarbonate pump is stimulated.[3]

Finally, urinary acidification was investigated with kidneys from potassium-adapted and potassium-depleted rats. Under conditions of an acute acid load, previous studies have shown that urine pH is higher than normal during potassium depletion and decreased in protassium loading [25]. But, because potassium depletion increases ammonia production, whereas potassium loading appears to inhibit this process [25], it is unclear whether the change in urine pH is the result of an intrinsic change in the hydrogen ion pump or relates solely to changes in urinary buffer excretion. In our studies with the isolated perfused kidney, ammonium excretion was comparable between potassium-loaded and potassium-depleted kidneys, and no differences in pH gradient were apparent. These findings do not provide any support for an intrinsic change in the hydrogen pump and suggest that the differences in urine phH between these two conditions in vivo result from alterations in ammonia production.

Summary. We have developed a technique using the isolated perfused kidney that, although indirect, seems to provide information relative to the hydrogen ion pump in the distal nephron. The data suggest that the characteristics of the pump are similar to that described in anuran epithelial membranes.

[2]The rate of ammonium excretion is somewhat surprising in view of the absence of amino acids from the perfusate. A sustained rate of production of this magnitude cannot be accounted for quantitatively by the endogenous amino acids contained in renal tissue [20]. Thus, the nitrogen source results from either albumin metabolism or catabolism of endogenous renal proteins. This question is unresolved at present.

[3]If both a hydrogen ion and bicarbonate secretory mechanism exist, net hydrogen ion secretion (that is, the difference between hydrogen ion and bicarbonate secretion) would determine urine pH. Thus, stimulation of a bicarbonate secretory mechanism would not necessitate the presence of an alkaline urine.

Futhermore, they indicate that the hydrogen ion transport system of the distal nephron adapts to chronic sodium bicarbonate loading. Additional studies with lower rates of buffer secretion are necessary to conclusively characterize the PMF of the pump, and those with heightened buffer delivery may elucidate the secretory capacity under varying conditions. Nevertheless, the present studies demonstrate that the characteristics of the pump, itself, are amendable to study in the intact mammalian kidney independent of the other variables that can alter distal hydrogen ion transport.

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